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Sexual dimorphism of the calcaneus in contemporary Cretans

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Highlights

- Sexual dimorphism of the calcaneus using metric data is investigated
- Student paired t-test was applied to examine bilateral asymmetry
- One-way ANOVA to select the variables that differ significantly between sexes
- High correlation found between the bone metrics and the known sex
- Standards for Athens are not suitable for Cretans due to high sex bias

ABSTRACT

During the past decade, several studies have been carried out using the calcaneus bone for sex estimation. This paper collected data using ten variables for metric characteristics of the calcaneus of 144 modern Cretans and examined their correlation with known sex. Secondly, the formulae developed by Peckmann et al. (2015) for modern Athenians was put to the test in order to investigate if it could be applied to this modern Cretan sample as well. Results showed a high correlation between the calcaneus metrics and the known sex of the individuals, however the formulae for Athenians do not seem to be suitable for the Cretans due to the high sex bias reported in this study. Thus, new standards were created for sex estimation from the calacanei in our sample. Bilateral asymmetry was noted in the majority of cases, thus formulae were developed for left, right and mean values. Maximum width (MAXW) was the variable that performed the best in the Cretan sample. Overall, the cross-validated accuracies for univariate and multivariate equations reached 84.2% with males most often correctly identified. The calcaneus was proved to be useful for sex estimation in this modern Cretan population. Further work will explore the suitability of the produced standards for other regions of mainland Greece and islands.

Keywords: Forensic Anthropology population data, Calcaneus, Sex estimation, Crete, Greece

INTRODUCTION

In the past several decades, forensic anthropology as well as bioarchaeological research have expressed a growing interest towards identifying the sex of an individual from its skeletal elements. Long bones, flat bones and the skull have been the subject of numerous studies based on different reference populations employing a vast variety of different measurements [1-5]. Amongst the sexually dimorphic bones of the human skeleton, the calcaneus, which is considered the most robust bone of the foot, is the focus of this study.

In humans, the calcaneus, or *heel bone* is a bone of the tarsus of the foot that constitutes the heel. The half of the bone proximal to the heel is the calcaneal tubercle. On its distal edge on either side are the lateral and medial processes, serving as the origins of the abductor hallucis and abductor digiti minimi muscles. The cuboid bone articulates with its anterior side, and on its superior side there are three surfaces for the articulation with the talus bone At the superior and anterior of the medial surface of the calcaneus, lies the sustentaculum tali, which serves as an attachment site. [6-8].

The calcaneus has long been acknowledged as an important skeletal element due to its role in locomotion and weight transmission and because it serves as an indicator of body size in modern humans and other hominids [9-11].

More specifically, Neanderthal calcanei have a relatively larger articular surface, longer calcaneal body and very projected sustentaculum tali compared to modern humans [10, 12, 13].

Even amongst humans, morphological variation of the calcaneus has been noted since 1931 by Wells [14] who described such differences between Bantu, Bushman and Europeans [15]. Decades later, Bunning and Barnett (1965) [16] reported variation on the number of talar articular facets of the calcaneus (1-3 facets) between British, Indians, Nigerians and indigenous people from Sri-Lanka. Ever since, the calcaneus has been the subject of several population specific studies for sex, stature and ancestry estimation [15, 17-26].

Sex estimation methods are traditionally based on osteometry but can also include more sophisticated tools in terms of data acquisition and analysis such as 3D reconstruction of bones from Computed Tomography or surface scans (white light, laser), geometric-morphometric methods, neural networks or decision trees. Following the current trends in forensic methodology radiographs [27] and CT scans of the calcaneus have also been employed for sex estimation [28].

This study focuses on the use of metric characteristics of the calcaneus in sex identification based on a contemporary sample from Crete, Greece. The calcaneus bone was used in the current study because it is often well preserved, as humans in modern societies tend to be found buried wearing shoes, which protect the heel from wear and post mortem erosion. Although standards for Greeks have been produced in the past [24], previous research suggests the existence of sex bias when the standards from mainland Greece are applied in the Cretan population [5]. The aim of this study is to explore the existence of sexual dimorphism in the Cretan calcaneus and to test the efficiency of the formulae produced for Athenians when applied in a contemporary collection from Crete. The results of this study will provide an additional sex estimation method of unidentified remains for the island of Crete.

MATERIALS AND METHODS

The skeletal material for this study was selected from the Cretan collection [29, 30]. The study population consists of individuals born in Crete between 1867 and 1956, and died between 1968 and 1998. Age and cause of death were obtained from the Heraklion City Hall census archives for only part of the skeletal material, while sex was inferred from the names written on the boxes that contained the remains and was cross-checked with pelvis morphology.

A total of 144 skeletons (76 males and 68 females) were measured according to standard osteometric techniques [31]. Both left and right calcanei were measured when present. Mean age for males was 68.9±13.3 years and for females 73.3±16.9 years. The following 10 measurements, easily assessed in skeletonized bodies, were taken: Maximum anterioposterior length (MAXL), Maximum height (MAXH), Cuboidal facet height (CFH), Body height (BH), Load arm length (LAL), Minimum transverse width (MINW), Maximum transverse width (MAXW), Dorsal articular facet breadth (DAFB), Dorsal articular facet length (DAFL) and Width of the sulcus calcanei (WSC) (Figure 1, Table 1). We followed Kim (2013) [40] and Peckmann (2015) [24] definitions and abreviations in this study. Measurements were taken using Digital Caliper 300mm/0.01mm with data output Mitutoyo. Specimens with known or obvious pathology and trauma were excluded.

Technical measurement error (TEM), relative TEM (rTEM) and the coefficient of reliability (R) were used to assess inter- and intra- observer error in a sample of 30 randomly selected bones [32].

Student paired t-test was used to test the existence of bilateral asymmetries for right and left bones. One-way ANOVA was used to select the variables that differ significantly between males and females ($p<0.05$). Data was submitted to discriminant function analysis to develop sex estimation formulae for the calcaneus.

A leave-one-out classification procedure was applied, in order to demonstrate the accuracy rate of the original sample and the one created by cross-validation. This procedure classifies all individual bones, by applying to each one of them the functions derived from all samples with the exception of one. Posterior probabilities of each individual were also calculated. Data analysis was carried out using the discriminant function subroutines of SPSS 22.0.

RESULTS

a) *Error estimates*

Thirty randomly selected bones were measured by two independent observers. The overall agreement was very high. DAFB was the variable with the higher error rate (R=0.94) (see Table 2).

b) Bilateral asymmetries

Table 3 shows the results of the paired student's T-test. According to these results there are statistically significant differences between the mean values of left and right calcaneus at $p<0.05$ for three variables (BH, LAL and DAFL). Therefore we decided to use the mean values of the measurements for developing the standards for the calcaneus.

c) Sex differences

Descriptive statistics of 10 measurements and the associated univariate F-ratio to measure the differences between the sexes for are shown in Table 4. The differences between the means in males and females were significant ($p<0.0001$) for all measurements except left and right and mean WSC. Thus, this variable is excluded from further analysis.

d) Test of the Peckmann et al., (2015) formulae.

We applied the standards produced by Peckmann et al. (2015) [24] in our sample. Six univariate and 4 multivariate functions were tested in total and the classification accuracy obtained in our sample in contrast with the original and cross-validated data can be seen in Table 5. Function 1 used all variables (MAXL, DAFL, LAL, MINB, MIDB, DAFB, BH, MAXH, CFH), Function 2 used length variables only (MAXL, LAL and DAFL), Function 3 used breadth variables only (MINB, MIDB, DAFB) and Function 5 was the stepwise multivariate discriminant function equation, using MAXL, DAFB and CFH. Classification accuracy for univariate functions ranged from 52.2% (MIDB) to 79% (MAXL). This is lower than the cross-validated accuracy reported in the original study, which ranged between 70% and 83%. Interestingly, sex bias for Cretans ranged between -15% to 95.5% as opposed to -13.3% to 20% in the original study. Similar results were obtained for the multivariate functions. The overall accuracy for Cretans ranged between 60% and 81% as opposed to the cross-validated accuracy for Athenians that ranged from 85% to 88%. Sex bias ranged from -20% to 75%. The most adequate equations would be MAXL and F2, which both give the same classification accuracy and the smallest possible sex bias for Cretans (-15% and -9% respectively). The equations that did not perform as well were MIDB and F3 with 95% and 75% sex bias, which clearly misclassified Cretan females in great extent.

e) DFA for Cretans

Univariate and multivariate DFA resulted in several formulae for Cretans. Table 6 presents the demarking points for single dimensions and the classification accuracy for original and cross-validated data for right, left and mean values of each variable. For example, a maximum length of the right calcaneus (RMAXL) smaller than 77.8 mm is designated as female while a length greater than that is designated as male. The most effective single dimensions, as demonstrated by direct discriminant analysis, were LMAXW (82.4%), followed by LLAL (82.1%), and DAFLMean (80.6%). Note that univariate functions with classification accuracy less that 80% were omitted from Table 6 as they are of limited value for forensic application [33].

The ten variables that were found to differ significantly between the two sex groups were submitted to direct and stepwise DFA resulting in five functions for the right (RF1-RF5), four for the left (LF1-LF4) and three for the mean measurements (MF1-MF3). All functions and the corresponding accuracies can be found in Table 7. The best formulae were found to be RF2, LF3 and MF3. Cross validation procedure results were very close to the original classification in all cases. Please note that only functions with over 80% classification accuracy for the original sample were included in Table 7.

DISCUSSION

Sex is one of the major factors that leads to a reliable identification of a person. Several studies have shown that sex estimation can be carried out using different anatomical parts of the skeleton, such as the pelvis, the skull and postcranial elements [2-5, 34]. The pelvis is the most commonly used bone, because its anatomical features lead to safer conclusions and it is believed to be population independent. This is mainly attributed to the fact that female pelvic feautures are shaped primarily to serve gestation and parturition [35-37]. When the pelvis is not present, which is a common occurrence in decomposed human remains, other elements of the human skeleton show variable degrees of sexual dimorphism as well, with the most common being the skull. Yet, recent studies suggest that sexual dimorphism is more pronounced in the postcranial skeleton [38].

All fifteen variables measured on the calcaneus of the Cretan population provided with statistically significant sex variations between males and females, suggesting that the calcaneus is sexually dimorphic in this population. Similar conclusions were cited for many different populations. Thesehowever, used a variety of measurements that are not always named or described consistently. For example, MAXW in Bidmos and Asala (2004) [18] is actually MIDB in Bidmos 2006 [39]. The variable that provided the highest accuracy rate in our data was the maximum width, and the one that performed the worst was the mean maximum length (Table 6). The most popular single variable among studies was maximum length (MAXL), which resulted in accuracies ranging from 76.25% for Modern Southern Italians [20] to 93.1% for modern Athenians [24]. The best single indicator for the Athens study was MinB which classified correctly 83.3% of the cross-validated data. Our study resulted in only 52% accuracy when the cut-off value from the Athens study was applied to our data with a sex bias towards males of 96%. This was the trend for all single variables as seen in Table 5. Clearly, the cut-off values from the Athens collection are inappropriate for application in individuals from Crete. The accuracies obtained from the use of single variables for Cretans ranged from 78.2% (MAXWMean) to 82.4% (LMAXW) for the original, whereas, for the cross-validated group, it ranged from 78% (MAXLMean) to 82.4% (LMAXW). These results indicate that there is a need for new metric standards for Cretans in order to address the high misclassification rates due to sex biases.

The formulae developed by Peckmann at al. (2015) [24] relying on the Athens collection, was tested on the Cretan collection in this study. Results showed from 60 to 81% accuracy in sex determination, with sex bias up reaching 75% (see Table 5), meaning that documented women often appear as men and vice versa. The only formulae that seems to be appropriate for application in the Cretan sample is F2 with 79% accuracy and -9% sex bias. The formulae developed for the Cretan sample gave accuracies ranging from 78% to 84.2% with a sex bias of less than 5% for cross-validated data. The best formulae was MF3, which used MAXH, BH and LAL (see Table 7). Thus, it is obvious that the formulae developed from the Athens Collection are not applicable to our Cretan sample, which is more representative of the original Cretan population. This behaviour can be partly attributed to population distinct features, such as genetics and occupational differentiation. In general, Athens population can be described as "urban" whereas the Cretan one has more of a "rural" character; that could be a possible explanation for the more pronounced anatomical features on the Cretan females. Females of rural regions tend to be involved in more manual labor compared to the Athenians, hence the robusticity of their bones, which classifies them as males according to the Athens standards. Similar results were obtained when formulae for the metacarpal bones from the Athens collection [41] were applied to the Cretan collection [5].

Bone size and bone mineral density (BMD) are subjected to a complex combination of genetic, lifestyle and nutritional control. BMD of the calcaneus peaks between 25 and 30 years old. Body weight, height, body fat, BMI are found to have a significant correlation with BMD of the calcaneus in both sexes in several studies [42]. Physical activity and high calcium intake were also found to increase BMD of the calcaneus [43]. This implies that any given factor of the abovementioned can have an effect on the size of the calcaneus in a given sample and subsequently can introduce bias in the expression of sexual dimorphism. Loss of BMD has been established in the Greek population with increasing age in normal and osteoporotic subjects [43-44]. The mean age of our sample for both males and females suggests that our subjects may have already suffered BMD loss at the time of death, which could potentially introduce bias in the study. Yet, none of the metric variables employed here showed significant correlation with age. Inversely, high daily activity producing increased load on the calcaneus increases BMD, which could explain the differences with the Athens sample assuming that Cretans were engaged in rural activities.

An alternative explanation of these discrepancies would be the sample effect. According to that, differences between populations in anatomy and other characteristics might be a result of a simple variation between different samples, instead of a population specific discrimination. In that case, other skeletal elements should be put to the test to collect evidence in order to justify that there is a significant biological difference between two populations. To date, this trend has been verified only for the metacarpal bones [5].

Sexual dimorphism of the calcaneus is widely discussed and analyzed in the literature for different modern populations. The purpose of this study was firstly to test the modern Cretans metric charateristics of the calcaneus, to examine their connection with the sex of and individual, and secondly to test the formulae developed for the Athenian sample, in order to find out whether they apply on the present or not. In addition, standards for Athenians generally misclassified Cretan females. This could be attributed to genetic or ocuppational differences or to the sampling efect. Future research could further explore this question which, however, exceeds the purpose of this work. The present study recommends that the Athenians' formulae are not applicable to the Cretans, and suggests new population specific standards for the calcaneus that can be directly applicable to forensic casework on the island of Crete.

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Figure 1. Superior, Inferior, Medial and Lateral views on a 3D printed Right Calcaneus bone. All ten measurements taken for this study are demonstrated using arrows.

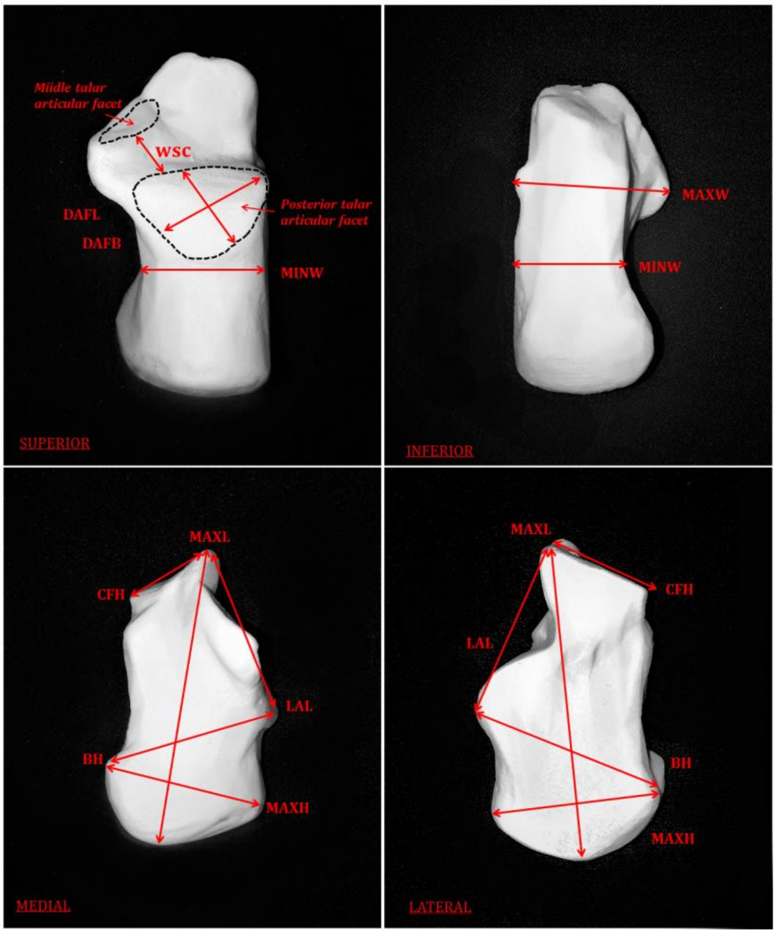

Table 1. Measurements and abbreviations

ABBREVIATION	DEFINITION	EXPLANATION
MAXL	maximum anterioposterion length	Linear distance between the most anterior point of the calcaneus and the most posterior point on the calcaneal tuberosity
MAXH	maximum height	Linear distance between the most superior and the most inferior points on the calcaneal tuberosity.
CFH	cuboidal facet height	Linear distance between the most superior and the most inferior points on the cuboidal articular facet.
BH	body height	Linear distance between the superior and the inferior surfaces of the body of the calcaneus taken in the coronal plane, at the midpoint between the most posterior point of the posterior articular facet and the most anterior point of the calcaneal tuberosity.
LAL	load arm length	Linear distance between the most anterior point on the calcaneus and the most posterior point on the posterior articular facet.
MINW	minimum transverse width	Minimum distance between the medial and lateral surfaces of the body of the calcaneus. (=MINB Peckmann)
MAXW	maximun transverse width	Linear distance between the most lateral point on the posterior articular facet and the most medial point on the sustentaculum tali. (=MIDB Peckmann)
DAFB	dorsal articular facer breadth	Linear distance from the most medial to the most lateral points on the posterior articular facet.
DAFL	dorsal articular facer length	Linear distance between the most posterior and the most anterior points on the posterior articular facet of the calcaneus.
WSC	width of the sulcus calcanei	Width between the middle and the posterior articular facet

Table 2. Intra-observer error is quantified using TEM,rTEM and R

	TEM	rTEM	R
MAXL	0.387	0.489	0.994
MAXH	0.402	0.893	0.990
BH	0.444	1.031	0.984
LAL	0.502	1.047	0.972
CFH	0.521	1.052	0.983
MINW	0.384	1.384	0.983
MAXW	0.516	1.042	0.985
DAFL	0.476	1.638	0.959
DAFB	0.423	1.399	0.940
WSC	0.278	4.879	0.957

Table 3. Billateral asymmetry of the Cretan calcaneus.

V	N	Mean	SD	T-value	P-value
RMAXL	123	78.17	5.30	0.2407	0.8102
LMAXL	123	78.15	5.29		
RMAXH	122	44.64	3.57	-0.5329	0.3084
LMAXH	122	44.74	3.67		
RCFH	124	23.70	2.12	-5.6829	0.5951
LCFH	124	23.75	2.09		
RBH	122	41.90	3.57	2.3725	**0.0000**
LBH	122	42.59	3.59		
RLAL	123	47.61	3.59	0.8483	**0.0192**
LLAL	123	47.35	3.65		
RMINW	119	26.87	2.36	0.9024	0.3980
LMINW	119	26.81	2.40		
RMAXW	124	48.14	3.53	2.2306	0.3687
LMAXW	124	48.03	3.51		
RDAFL	122	28.66	2.43	1.0713	**0.0275**
LDAFL	122	28.45	2.44		
RDAFB	124	29.75	2.41	-0.9836	0.2862
LDAFB	124	29.65	2.45		
RWSC	122	5.618	1.18	-1.0228	0.3273
LWSC	122	5.69	1.15		

Table 4. Mean differences between males and females for right, left and mean values of all variables

V	Sex	N	Mean	SD	F-Value
RMAXL	Males	75	81.04	4.22	81.23
	Females	67	74.64	4.23	
LMAXL	Males	66	81.13	4.28	69.76
	Females	67	74.80	4.45	
MAXLMean	Males	63	81.11	4.34	60.53
	Females	60	75.06	4.30	
RMAXH	Males	76	46.62	3.25	61.69
	Females	68	42.72	2.64	
LMAXH	Males	66	46.74	3.45	56.18
	Females	66	42.71	2.69	
MAXHMean	Males	63	46.54	3.34	48.32
	Females	59	42.71	2.67	
RCFH	Males	75	24.71	1.88	48.56
	Females	67	22.54	1.83	
LCFH	Males	66	24.74	1.73	46.52
	Females	67	22.53	1.99	
CFHMean	Males	63	24.68	1.71	36.99
	Females	59	22.71	1.88	
RBH	Males	76	43.72	3.24	61.62
	Females	67	39.88	2.50	
LBH	Males	67	44.57	3.32	60.90
	Females	68	40.64	2.48	
BHMean	Males	64	44.16	3.31	57.11
	Females	60	40.20	2.43	
RLAL	Males	75	49.46	3.04	73.44
	Females	66	45.34	2.61	
LLAL	Males	66	49.47	2.99	80.61
	Females	68	44.98	2.79	
LALMean	Males	63	49.53	3.04	66.83
	Females	59	45.28	2.68	
RMINW	Males	75	27.99	2.31	43.94
	Females	66	25.63	1.84	
LMINW	Males	67	27.93	2.27	46.41
	Females	67	25.47	1.91	
MINWMean	Males	64	27.96	2.23	40.29
	Females	59	25.63	1.80	
RMAXW	Males	72	49.87	3.22	56.40
	Females	65	46.02	2.72	
LMAXW	Males	65	50.14	3.00	71.25
	Females	66	45.87	2.79	
MAXWMean	Males	61	49.96	3.08	52.52
	Females	58	46.12	2.68	

RDAFL	Males	76	29.86	2.04	60.35
	Females	67	27.28	1.93	
LDAFL	Males	67	29.85	1.94	72.47
	Females	69	26.92	2.08	
DAFLMean	Males	64	29.89	2.00	62.94
	Females	60	27.13	1.91	
RDAFB	Males	76	30.81	2.22	50.26
	Females	67	28.31	1.95	
LDAFB	Males	67	30.98	2.20	69.12
	Females	67	28.10	1.79	
DAFBMean	Males	64	30.95	2.10	54.07
	Females	58	28.31	1.84	
RWSC	Males	76	5.64	1.18	0.27
	Females	68	5.53	1.22	
LWSC	Males	67	5.73	1.18	0.21
	Females	68	5.63	1.14	
WSCMean	Males	64	5.70	1.12	0.24
	Females	60	5.60	1.08	

Table 5. Accuracy of the formulae developed on the Athens collection on the Cretan sample

	Cretans					Athens					
	Male (N=67)		Female (N=67		Total	Original			Cross-Validated		
	N	%	N	%	%	M	F	Tot	M	F	Tot
MAXL	48	71.6	58	86.6	79.1	80.7	86.3	83.5	78.6	86.7	82.7
LAL	31	46.3	64	95.5	70.9	79.5	87.5	83.5	78.6	86.7	82.7
MIDB	67	100.0	3	4.5	52.2	78.4	85.0	81.7	80.0	86.7	83.3
DAFB	45	67.2	59	88.1	77.6	81.8	81.3	81.6	66.7	80.0	73.3
BH	27	40.3	62	92.5	66.4	79.5	83.8	81.7	80.0	60.0	70.0
MAXH	36	53.7	54	80.6	67.2	80.7	82.5	81.6	86.7	78.6	82.6
F1	66	98.5	24	35.8	67.2	86.4	93.1	89.5	91.7	83.3	87.5
F2	50	74.6	56	83.6	79.1	85.2	87.5	86.3	85.7	86.7	86.2
F3	65	97.0	15	22.4	59.7	77.8	90.3	83.7	76.9	92.3	84.6
F5	47	70.1	61	91.0	80.6	88.9	87.5	88.2	85.7	86.7	86.2

Table 6. Classification accuracy and demarking point left, right and mean measurements of the Cretan calcaneus

		Original					Cross-validated				
V	**Demarking value**	**Males**		**Females**		**Total**	**Males**		**Females**		**Total**
		N	**%**	**N**	**%**	**%**	**N**	**%**	**N**	**%**	**%**
RMAXL	77.84	61/75	81.3	53/67	79.1	80.3	61/75	81.3	53/67	79.1	80.3
LLAL	51.67	56/66	84.8	54/68	79.4	82.1	56/66	84.8	54/68	79.4	82.1
LALMean	47.41	51/63	81.0	47/59	79.7	80.3	51/63	81.0	47/59	79.7	80.3
LMAXW	*48.00*	*53/65*	*81.5*	*55/66*	*83.3*	*82.4*	*53/65*	*81.5*	*55/66*	*83.3*	*82.4*
DAFLMean	28.51	55/64	85.9	45/60	75.0	80.6	55/64	85.9	45/60	75.0	80.6

Table 7. Discriminant functions and classification accuracy

											Original					Cross-validated				
											Males		**Females**		**Total**	**Males**		**Females**		**Total**
	MAXL	**MAXH**	**CFH**	**BH**	**LAL**	**MINW**	**MAXW**	**DAFL**	**DAFB**	**CONSTANT**	**N**	**%**	**N**	**%**	**%**	**N**	**%**	**N**	**%**	**%**
RF2	0.16	0.16								-19.36	65/75	86.7	53/66	80.3	83.7	64/75	85.3	53/66	80.3	83.7
RF3	0.14	0.10		0.03					0.11	-20.12	64/75	85.3	55/65	84.6	85.0	63/75	84.0	54/65	83.1	83.6
RF4	0.13	0.12						0.15		-20.15	63/75	84	55/65	84.6	84.3	63/75	84.0	53/65	81.5	82.9
RF5				0.13	0.14			0.20		-18.00	64/75	85.3	52/66	78.8	82.3	64/75	85.3	52/66	78.8	82.3
LF2					0.13			0.20	0.21	-18.20	54/66	81.8	55/66	83.3	82.6	53/66	80.3	52/66	78.8	80.0
LF3		0.08			0.19		0.13			-18.94	53/64	82.8	56/66	84.6	83.8	53/64	82.8	56/66	84.6	83.8
LF4	0.02	0.04			0.14		0.09		0.18	-19.43	53/64	82.8	55/64	85.9	84.4	52/64	81.3	53/64	82.8	82.0
MF1	0.11	0.08	0.11	0.09						-18.77	54/62	87.1	45/54	83.3	85.3	53/62	85.5	44/54	81.5	83.6
MF2	0.05	0.10		0.05	0.17					-18.75	53/62	85.5	48/57	84.2	84.9	53/62	85.5	46/57	80.7	83.2
MF3		0.10		0.06	0.23					-18.11	52/62	83.9	50/58	86.2	85.0	52/62	83.9	49/58	86.2	**84.2**